abandoned, which is a file wrapper continuation of patent application Serial No. 07/271,609 filed November 15, 1988, now abandoned, as follows:

In the Specification

On page 4, under the heading Brief Description of the Drawings, please delete page 4, line 5 through page 12, line 12 and insert:

---Figure 1 presents an outline of CISS hybridization for specific staining of human chromosomes.

Figures 2A-2F show suppression from cross-reacting sequences within a chromosome 7-derived DNA library by different concentrations of human competitor DNA. Biotin-labeled chromosome 7 DNA inserts (20 μg/ml) were prehybridized for 20 minutes with human genomic DNA prior to hybridization with metaphase chromosome spreads and detection with FITC-labeled avidin. Genomic salmon DNA was added to each sample to adjust the final DNA concentration to 1.0 mg/ml (see the text for details). The arrows mark the target chromosome 7 and the arrowheads mark additional strong signals on non-7 chromosomes. All negatives printed were exposed and developed under identical photographic conditions.
Figure 2A shows prehybridization with 0 μg/ml human competitor DNA.
Figure 2B shows prehybridization with 50 μg/ml of human competitor DNA.
Figure 2C shows prehybridization with 100 μg/ml of human competitor DNA.
Figure 2D shows prehybridization with 200 μg/ml of human competitor DNA.
Figure 2E shows prehybridization with 1000 μg/ml of human competitor DNA.
Figure 2F is the same as Figure 2E except that the metaphase spread is post-stained with DAPI.

Figures 3A-3D show the effect of pre-annealing time on the specificity and strength of the hybridization signal. Biotin-labeled chromosome 7 DNA inserts (20 μg/ml) were preannealed with 200 μg/ml human competitor DNA for different times prior to hybridization to metaphase chromosomes.
Figure 3A shows preannealing for 0 minutes.
Figure 3B shows preannealing for 2 minutes.
Figure 3C shows preannealing for 5 minutes.

Figure 3D shows preannealing for 20 minutes.

Figure 4A show decoration of chromosome 1 in normal human lymphocytes. The signal of chromosome 1 was amplified by the sandwich technique of Pinkel et al. (1986).
Figure 4B shows decoration of chromosome 7 in normal human lymphocytes.
Figure 4C shows decoration of chromosome 4 in normal human lymphocytes.
Figure 4D shows decoration of chromosome 18 in normal human lymphocytes.
Figure 4E shows decoration of chromosome 13 in normal human lymphocytes. Only the chromosome 13 insert DNA pool shows significant cross-hybridization to other chromosomes after the prehybridization suppression step.
Figure 4F shows decoration of chromosome 20 in normal human lymphocytes. The detection of chromosome 20 was done with the entire chromosome library (including λ phage arms) and detected with avidin-alkaline phosphatase using nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate (NBT-BCIP) as the enzyme substrate mixture.

Figures 5A-5F show chromosome domains in human lymphocyte nuclei delineated by preannealed chromosome library DNA inserts.
Figure 5A and Figure 5B show domains for chromosome 1. Hybridization to acetic acid-methanol fixed nuclei was detected by fluorescein isothiocyanate (FITC)-conjugated avidin.
Figure 5C and Figure 5D show domains for chromosome 7. Hybridization to acetic acid-methanol fixed nuclei was detected by fluorescein isothiocyanate (FITC)-conjugated avidin. A predominant staining of the centromere region is seen within the chromosome 7 domains, reflecting preferential hybridization of the chromosome 7-specific alphoid DNA repeat; a similar signal distribution on metaphase chromosomes was also observed in the particular experiment.
Figure 5E shows domains for chromosome 18. Hybridization to acetic acid-methanol fixed nuclei was detected by fluorescein isothiocyanate (FITC)-conjugated avidin.
Figure 5F shows domains for chromosome 18. Hybridization to acetic acid-methanol fixed nuclei was detected by alkaline phosphatase-conjugated avidin.

Figure 6A shows chromosomal in situ suppression (CISS) hybridization of chromosome 1 inserts to metaphase spreads of the TC 620 glioma cell line detected with FITC-avidin. Figure 6B is the same as Figure 6A except that the metaphase

spreads are post-stained with 4,6-diamidino-2-phenylindole dihydrochloride (DAPI). TC 620 show two apparently complete 1 chromosomes (small arrows in B) and two marker translocation chromosomes (arrowheads) specifically decorated by these inserts. One of the two marker chromosomes contains a 1p (lower left), the other a 1q arm (lower right); the 1p terminal (relatively GC rich region) in the two normal chromosomes and submetacentric marker is less completely delineated. Also, the 1q12 regions here show little decoration in contrast to most experiments. X950.

Figure 6C shows chromosomal in situ suppression (CISS) hybridization of chromosome 1 inserts to metaphase spreads of the TC 593 glioma cell line detected with FITC-avidin.

Figure 6D is the same as Figure 6C except that the metaphase spreads are post-stained with 4,6-diamidino-2-phenylindole dihydrochloride (DAPI). Typical TC 593 metaphase spreads show six specifically decorated chromosomes. Three acrocentric marker chromosomes all with truncation of 1p show particularly intense fluorescence of repeats that localize to 1q12 (arrows in C). In two of these, 1q arms appear to be complete, while a major deletion is obvious in the third (arrow in D). A fourth decorated chromosome (small arrowhead in C, D) again shows a major deletion of the distal part or 1q, but has retained an apparently complete 1p arm. A fifth submetacentric chromosome (large arrowhead) contains an apparently complete 1p arm; the DNA of its short arm is not identified. Note the similarity of this marker to one of the marker chromosomes of TC 620 (1p) described above. The sixth entirely decorated chromosome is an iso (1p) as demonstrated by DAPI-binding (open arrows). X 1200.

Figures 7A-7J show CISS hybridization of chromosome 4 library inserts detected with FITC-avidin.

Figure 7A shows interphase nuclei of TC 593. Note that the two apparently complete interphase domains are widely separated.

Figure 7B shows interphase nuclei of TC 593. Note that the two apparently complete interphase domains are close to each other.

Figure 7C shows interphase nuclei of TC 593. Note that the two apparently complete interphase domains are widely separated.

Figure 7D shows interphase nuclei of TC 620 showing four chromosome 4 interphase domains of largely different sizes.

Figure 7E shows metaphase spread of TC 593 showing two apparently complete 4 chromosomes, and a small decorated region (arrow) in a submetacentric chromosome. This marker with translocated 4 sequences was observed in about 30% of the spreads.

Figure 7F shows metaphase spread of TC 620 showing one apparently complete chromosome 4 and three translocation markers (t) containing different amounts of chromosome 4 material.
Figure 7G shows double hybridization of biotinylated chromosome 7 inserts and an aminoacetylfluorene (AAF)-modified 7-specific alphoid repeat. Chromosome 7 inserts depict five entirely decorated metaphase chromosomes. Four of them are complete 7 chromosomes, the fifth (arrow) is an iso (7p) (see Figure 3E).
Figure 7H shows the same field as G showing AAF-7 alphoid signals on only four decorated chromosomes; no signal is detected on the iso (7p).
Figure 7I shows an interphase nucleus of TC 593 showing five domains delineated by chromosome 7 inserts. The arrow represents the iso (7p) marker in interphase.
Figure 7J shows the same field as I showing that four of the domains are labeled by 7 alphoid probes.

Figure 8A shows CISS hybridization of library inserts of chromosome 7 to metaphase spreads of TC 620 glioma cells detected with FITC-avidin. x 875. Three apparently normal 7 chromosomes and an additional translocation chromosome containing 7 sequences are indicated by large arrowhead.
Figure 8B is the same as for Figure 8A except counter-stained with DAPI. DAPI-stained complete chromosomes are indicated by small arrowheads. Other studies (see the text) indicated a translocation of 7pter-q11 in the marker chromosome (large arrowhead).
Figure 8C shows CISS hybridization of library inserts of chromosome 18 to metaphase spreads of TC 620 glioma cells detected with FITC-avidin. Two apparently complete 18 chromosomes and a truncated minute chromosome (large arrowhead) are shown.
Figure 8D is the same as for Figure 8C except counter-stained with DAPI.
Figure 8E shows metaphase spread from pseudotetraploid TC 593 cells showing five chromosomes highlighted by 7 library inserts. The metacentric chromosome (m) represents the iso (7p) marker typical for this line (see also Figure 2G). Insert chromosomes (small arrows) show DAPI-stained normal and metacentric 7 chromosomes. The landmark band 7q21 and a block of constitutive heterochromatin at 7q11 are both prominent on the normal chromosome 7 insert (arrows) but not present on the marker chromosome. Instead both arms of the latter show a mirror-like staining pattern with a faint distal band at 7p21.

Figure 8F shows CISS hybridization of library inserts of chromosome 18 to metaphase spreads of TC 593 glioma cells detected with FITC-avidin. Four decorated 18 chromosomes are shown.
Figure 8G is the same as for Figure 8F except counter-stained with DAPI. Three decorated 18 chromosomes are clearly translocated.

Figure 9 is a summary chromosome idiogram of complete and aberrant chromosomes detected by CISS hybridization of library inserts of chromosome 1, 4, 7, 18 and 20 in glioma cell lines TC 620 (left) and TC 593 (right). G-bands (black) are shown with approximate breakpoints suggested by our data; the shaded areas with a wavy pattern are from other chromosomes that constitute part of the marker translocation chromosomes. The black dot beside two of the TC 620 translocated 4 segments indicates that the assignment of the chromosome 4 material is based on circumstantial evidence (e.g., size measurements). A small translocation of chromosome 18 material in ca. 20% of TC 593 metaphase spreads (+) also could not be further identified. Note the over-representation of 7p in both cell lines.

Figures 10A-10H show representative nuclei reflecting metaphase abnormalities in glioma cell lines (cf. Figs 1-3).
Figure 10A: Detection of the 7p translocation (t) in a prophase TC 620 nucleus. X 1,000.
Figure 10B: Detection of five well-separated chromosome 7 domains in interphase TC 593. X 1,240.
Figure 10C: Detection of two large and one very small 18 domains (indicated by arrowheads) in interphase TC 6. X 1,450.
Figure 10D: Detection of four chromosome 18 domains in interphase TC 593; one of these signals (arrows) appears smaller. X 1,450.
Figure 10E: Detection of four chromosomal 1 domains detected in interphase TC 620 (cf. Figure 1A, 1B). X 1,200.
Figure 10F: Detection of at least five chromosome 1 domains in interphase TC 593; one (arrow head) is appreciably smaller than the others. X 1,250.
Figure 10G: CISS hybridization of a metaphase spread of TC 593 with chromosome 18.
Figure 10H: The same as for 10G except counter-stained with DAPI. The technically poor metaphase spread still highlights four distinct chromosomes bearing 18 sequences. X 1,000.

Figures 11A-11E are graphic representations of the interphase and/or metaphase counts of chromosomes 7, 22 and 4 by CISS hybridization. Interphase counts were performed on 150 nuclei of well-hybridized preparations. For metaphase counts > 25 complete DAPI-stained spreads were evaluated.

Figure 11A: Counts of 7 specific alphoid repeats (white columns) compared to 7 library inserts (shaded columns) from interphase nuclei of phytohemagglutinin-stimulated human lymphocytes (46, XY).

Figure 11B: The same as for Figure 11A except from TC 620 interphase nuclei (7-specific alphoid repeats) and metaphase spreads (7 library inserts).

Figure 11C: The same as for Figure 11A except from TC 593 interphase nuclei (7-specific alphoid repeats) and metaphase spreads (7 library inserts).

For Figures 11 A-C: High stringency hybridization (see Materials and Methods) of 7 alphoid repeat was used to avoid cross-hybridization to other chromosomes. In cases of double hybridization with both 7 library inserts and alphoid repeat (shown in Figure 2 G-I) standard conditions with 50% formamide were sufficient to avoid cross-hybridization, possibly due to the presence of human competitor DNA.

Figure 11D: Counts of chromosome 22 (library inserts) in metaphase spreads of TC 620 (black columns) and TC 593 (shaded columns). For comparison, CISS hybridization was simultaneously performed with 7 library inserts in these experiments as an internal control (see C, D and Fig. 7).

Figure 11E: Interphase counts (white columns) and metaphase counts (shaded columns) compared in TC 593 hybridized with chromosome 4 inserts. Note the ratio of signal preparations 2:3 are the same in metaphase and interphase.

Figure 12 shows a TC 620 metaphase spread after double hybridization with inserts from chromosome 7 and 22 (both labeled with biotin and detected with avidin-FITC). Two strongly decorated 22 chromosomes (arrows), three complete 7 chromosomes and the metacentric marker chromosome containing 7pter-q11 are also seen.

Figure 13 is a graphic representation of the relative size of decorated normal and aberrant chromosomes 4, 7 and 18 in typical metaphase spreads (n=24) from glioma cell lines TC 593 and TC 620. Individual areas were normalized so that a complete chromosome is represented by an area of 1 (see legend to Table 1). The total added signals reflect the number of specific chromosome equivalents present. The white regions correspond to apparently normal chromosomes, the black regions indicate small

free chromosome segments entirely decorated by specific library inserts, and translocated segments are shade. One of the three translocated 18 chromosomes in TC 593 represents a complete chromosome by this measurement (indicated by the black dot), while the two other translocations are slightly smaller, possibly due to the small sample size.

Figures 14A-14O show specific labeling of human chromosome 21 by CISS hybridization with biotinylated DNA probe sets.

Figure 14A: Hybridization of plasmid pPW519-1R (6 kb insert) to a normal lymphocyte metaphase spread. Signals are located on the termini of 21q (see DAPI-stained chromosomes in Inset) as verified by DAPI banding (not shown).

Figure 14B: Hybridization of the 94 kb plasmid pool probe set to normal human lymphocyte metaphase spread. The terminal band 21q22.3 is specifically labeled.

Figure 14C: The same as for Figure 14B except that hybridization was to normal human lymphocyte nuclei.

Figure 14D: Hybridization of the 94 kb probe set to trisomy 21 (47, +21) lymphocyte metaphase spreads.

Figure 14E: Hybridization of chromosome 21 library DNA inserts to trisomy 21 (47, +21) lymphocyte metaphase spreads. Three chromosomes 21 are entirely delineated by the library inserts; additional minor signals (see the text) are indicated by arrowheads (also in Figure 14G).

Figure 14F: Hybridization of chromosome 21 library DNA inserts to trisomy 21 (47, +21) lymphocyte interphase nuclei (compare with 14E).

Figure 14G: Hybridization of chromosome 21 library DNA inserts to trisomy 21 (47, +21) lymphocyte interphase nuclei.

Figure 14H: Hybridization of chromosome 21 library DNA inserts to trisomy 21 (47, +21) lymphocyte interphase nuclei.

Figure 14I:Hybridization of chromosome 21 library DNA inserts to trisomy 21 (47, +21) lymphocyte interphase nuclei.

Figure 14J: Hybridization of chromosome 21 library DNA inserts to trisomy 21 (47, +21) lymphocyte interphase nuclei.

Figure 14K: Hybridization of the 94 kb probe set to chorionic villi (CV) cell interphase nuclei.

Figure 14L: Hybridization of the 94 kb probe set to CV cell metaphase spreads.

Figure 14M: Hybridization of the 94 kb probe set to CV cell metaphase spreads.